**Supplemental materials**

This study was performed on the same material as used in our previous studies (Ishunina et al., 2004). Clinico-pathological information is presented in the Table 1. The demented patients were clinically assessed and diagnosed as ‘probable VD” in accordance with the NINDS-AIREN criteria (Roman et al., 1993) and as ‘probable AD’ following the NINCDS-ADRDA criteria (McKhann et al., 1984). Controls had no signs of cognitive impairment and no history of any neurological or psychiatric disorder and were matched for age, sex, and fixation time with AD and VD cases. Neuropathological examination of AD patients assessed the distribution of neurofibrillary tangles over the brain according to the classification of Braak (Braak and Braak, 1991). Jellinger and [Bancher (1997)](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bancher%20C%5BAuthor%5D&cauthor=true&cauthor_uid=9330987) showed by multivariant analysis significant correlations between psychostatus and both the CERAD criteria and Braak staging of neuritic Alzheimer-type lesions. In addition, we recently reported a high correlation (0.874, p<0.001) between the clinical Reisberg scale and Braak scores (Zhu et al., 2016). On the basis of Braak’s analysis a score of 0-6 was assigned to the patients: the AD subjects had a score of 5-6, VD cases of 1-2 and the controls of 0-1. Neuropathological examination of VD patients revealed dilated ventricles, multiple hemorrhages and their remnants, necrotic lesions and atrophy of gyri and adjacent white matter, rarefaction and maceration of the white matter, lacunae, état criblé in the caudate nucleus, putamen, pallidum and thalamus. ‘‘Old’’ and ‘‘recent’’ infarctions in the VD group were localized in the temporal, parietal, occipital and occasionally prefrontal cortex, in the cingulate gyrus, insula, caudate nucleus, putamen, pallidum, capsula interna and externa, cerebellum and pons. None of the vascular lesions was found in the studied hypothalamic nuclei. It should also be noted that in all VD cases only slight senile involutive changes were present, and Braak stage did not exceed II in contrast to AD patients.

**Immunocytochemical staining**

Immunocytochemical staining of the ERα was performed as described earlier (Ishunina et al., 2003). Specificity of the staining was shown in (Kruijver et al., 2002). Briefly, following deparaffinization in xylene and graded ethanols, rinsing in Tris containing buffered saline (TBS) (pH 7.6), waterbath pre-treatment in 0.05M Tris-HCl buffer (pH 7.6) for 30 min at 900C and washing in milk-TBS for 1 hour at room temperature (RT) the sections were incubated with a primary polyclonal rabbit anti-ERα antibody that recognises the carboxy terminus epitope of the ERα (Santa Cruz, cat # sc-542) diluted 1: 100 in sumi-milk (0.25% gelatine and 0.5 ml Triton X-100 and 5% of milk powder in 100 ml TBS, pH 7.6) for 1 hour at RT and at 40C overnight. The next day sections were washed in milk-TBS, incubated with secondary biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA) 1:200 in sumi-milk for 1 hour at RT; washed in TBS and incubated with avidin-biotin complex (ABC) (Elite kit, Vector Laboratories, Burlingame, CA) 1:400 in sumi for 1 hour at RT. The final step was the incubation in Tris-HCl containing 0.05% 3,3' diaminobenzidine, 0.01% H2O2 and 0.3% nickel ammonium sulphate. Following washing in Tris-HCl buffer sections were dehydrated in graded ethanols, cleared in xylene and coverslipped with Entellan mounting medium. Morphometric analysis was performed using the ImageJ program (Ishunina, 2015).

**Table 1. Clinico-pathological information for patients with VD, AD and controls**

# Patient age sex fix bw pmd BS APOE Cause of death

**Vascular dementia patients**

1) 61 f 34 990 4:45 1O 33 Vascular dementia, cardiac arrest

2) 64 m 33 1662 5:25 nr nr Vascular dementia, cause of death unknown

3) 71 f 73 1274 3:45 1 nr Vascular dementia, aspiration pneumonia

4) 72 m 40 1365 9:00 nr 32 Vascular dementia, pneumonia

5) 77 f 37 971 4:40 1A 22 Vascular dementia, cardiac asthma

6) 78 m 30 1467 3:55 1 32 Vascular dementia, pneumonia

7) 80 f 32 1106 4:25 1 22 Vascular dementia, pneumonia

8) 81 m 33 1330 3:55 1 33 Vascular dementia

9) 86 m 46 1251 7:30 1 33 Vascular dementia, complications of a fall

10) 88 f 29 1154 3:20 2 32 Vascular dementia, dehydration, cachexia

11) 88 f 32 1047 5:00 2 42 Vascular dementia, acute heartfailure

**Control patients**

1) 60 f 87 nr 8.00 0 nr ovarian carcinoma

2) 61 f 28 1311 5:15 0 32 glioblastoma, coma

3) 66 m 49 1461 <41:00 nr nr adenocarcinoma of the stomach, septic shock

4) 69 f 31 1264 6:15 1 33 cardiogenic shock

5) 72 m 34 1383 6:45 0 43 heart failure

6) 76 f 270 1226 7:35 nr nr ovarian adenocarcinoma, extensive

metastases, lung edema, hydronephrosis

7) 78 m 79 1340 <52:50 nr nr cardio-pulmonary insufficiency, lung oedema

8) 80 f 34 1087 6:15 2B 42 angina pectoris, diabetes mellitus,

dehydration

9) 82 m 63 nr <89:00 1O nr lung carcinoma, bronchopneumonia

10) 85 f 28 925 5.17 0 33 pneumonia

11) 89 f 30 1220 6:25 1B 43 aspiration pneumonia

**Alzheimer’s disease patients**

1) 60 f 29 1060 3:45 6 33 Alzheimer’s disease, sudden death

2) 63 m 30 1350 5:00 6 33 Alzheimer’s disease, pneumonia

3) 72 f 69 958 4:00 5 nr Alzheimer’s disease, bronchopneumonia

4) 72 m 26 1520 5:15 5 43 Alzheimer’s disease, pneumonia

5) 78 f 28 959 4:25 5 43 Alzheimer’s disease, cachexia

6) 79 m 47 959 5:45 4 43 Alzheimer’s disease, pneumonia

7) 80 f 57 1139 2:15 6 43 Alzheimer’s disease, sudden death

8) 82 m 32 1317 4:15 5 44 Alzheimer’s disease, pneumonia

9) 85 m 29 1155 6:15 4 33 Alzheimer’s disease, kidney insufficiency

10) 87 f 27 1115 6:20 5 43 Alzheimer’s disease, acute heart failure

11) 88 f 20 1144 3:40 6 43 Alzheimer’s disease, cardiac decompensation

Abbreviations: m - male, f - female, Pmd - postmortem delay (in hours:minutes), fix - fixation time (in days), Bw - brain weight (in grams), APOE - APOE genotype, BS - Braak stage (numerical for neurofibrillar tangles and alphabetical for amyloid), nr - not recorded.

Postmortem delay did not influence the size of neuronal perikarya (r = -0.189) or their nucleus (r = -0.435) as shown by the absence of a significant correlation in Spearman’s linear regression analysis. Moreover, cases with most prominent postmortem delay had mean values within the main range of their group.

Postmortem delay appeared not to influence the number of neurons with strong (r = -0.451), weak (r = 0.257) or negative (r = 0.382) ERα staining according to Spearman’s linear regression analysis.

There were no statistically significant gender differences (p>0.05) and no significant correlations with age for any of the parameters within any of the studied groups (p>0.05).

The nuclear/cytoplasmic ratio did not change in either the VD or the AD in any of the studied nuclei (p>0.05). However, in all groups (VD, AD, Controls) this ratio was prominently higher in the MMN than in the TMN (p˂0.01).

**Table 2**. Mean +/- SEM values of neuronal nuclear and perikaryal profile in the posterior hypothalamus

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group** | **MMN** | | **TMN** | |
| S nucleus, µm2 | S perikaryon, µm2 | S nucleus, µm2 | S perikaryon, µm2 |
| **VD** | 72,75±2,97 1 | 202,87±14,82 3 | 94,6±8,98 | 348,7±29,98 |
| **AD** | 66,2±3,6 2 | 170,9±11,24 | 94,7±4,98 4 | 341,18±20,08 5 |
| **Control** | 53,13±4,04 1,2 | 149,8±10,1 3 | 108,3±4,89 4 | 404±13,74 5 |

Abbreviations: MMN – the medial mamillary nucleus, TMN – the tuberomamillary nucleus, S – surface area, VD – vascular dementia, AD – Alzheimer’s disease, Control – non-demented control groups;

1,2,3,5 - p˂0.01; 4 - p˂0.05.

**Table 3**. Mean +/- SEM values of neuronal nuclear and perikaryal profiles depending on the level of ERα nuclear staining in the MMN.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ERα nuclear staining** | **VD** | | **AD** | | **Control** | |
| nucleus, µm2 | perikaryon, µm2 | nucleus, µm2 | perikaryon, µm2 | nucleus, µm2 | perikaryon, µm2 |
| Strong (+++) | 75,7±2,91 | 160,3±6,54,5 | 62,5±1,68,9,10 | 170,4±6,3  13,14 | 45,12±2,4  17 | 135,4±6,2 20,21 |
| Moderate (++) | 82±1,762 | 204,9±6,74,6 | 74,8±1,48,11 | 205±5,413,  15,16 | 63,8±1,6  17,18,19 | 166,4±5,4 |
| Weak (+) | 78,7±3,13 | 191,2±7,15,7 | 55,1±2,29,12 | 148,3±11,1  15 | 53,9±4,1  18 | 169,2±10,3 20 |
| Negative (-) | 63,3±2,51,2,3 | 163,7±6,46,7 | 46,4±1,810,  11,12 | 129,9±5,2  14,16 | 47,9±1,9 19 | 154,7±6,3 21 |

Abbreviations: MMN – the medial mamillary nucleus, VD – vascular dementia, AD – Alzheimer’s disease, Control – non-demented control groups; indicated differences between groups - p˂0.01.

**Figure 1**

Fig. 1.tif

**Figure 1.** Microphotographs of the ERα staining in vascular dementia (VD) (A,B,C), Alzheimer’s disease (AD) (D,E,F) and non-demented control (NDC) (G,H,I) groups. Note differences in the size of neuronal nuclei and perikarya in relation to the intensity of the nuclear ERα staining: strong +++ (A,D,G), moderate (B,E,H) and negative (C,F,I). Examples of the strong nuclear ERα staining are presented in D and G (dark black nuclei). (J) Examples of the weak intensity ERα staining Objective x 40.

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